

# Therapeutic Response of Human Tumor Xenografts in Athymic Mice to Doxorubicin<sup>1</sup>

Rinaldo C. Giuliani,<sup>2</sup> Karimullah A. Zirvi,<sup>3</sup> and Nathan O. Kaplan

Department of Chemistry, Q-058, Cancer Center, University of California, San Diego, La Jolla, California 92093

## ABSTRACT

In order to establish the usefulness of the human tumor-nude mouse system as a predictive screen for anticancer agents, 17 tumors (3 breast, 3 colon, 3 lung, 3 melanoma, 2 ovary, 1 prostate, 1 sarcoma, and 1 larynx), serially transplantable in athymic mice, were used to study antitumor activity of doxorubicin (Adriamycin). BALB/c nude mice were treated i.v. on a weekly basis for 3 to 4 weeks, starting when the tumor volume became relatively large (advanced stage of tumor treatment). All the tumors except lung tumor T 293 showed a 90 to 100% take rate and stable growth.

Doxorubicin, at dose levels of 6 and 10 mg/kg/injection i.v. every week for 3 weeks, showed significant activity against all the three breast tumors studied. As was expected on the basis of clinical data, doxorubicin showed no antitumor activity against the three different colon tumors. In the case of lung tumors, statistically significant activities against oat cell carcinoma T 293 and epidermoid carcinoma T 222 were observed. In contradiction to clinical data, doxorubicin was found to have significant activity against various melanomas studied and against ovarian tumor T 17. Experimental results obtained using doxorubicin against prostate, sarcoma, and larynx tumors also parallel the reported clinical data.

## INTRODUCTION

Human tumors transplanted into athymic mice (nude) do not completely represent the real human situation but are more closely akin to the human situation than are the murine tumors used in primary screening studies; thus, they provide a unique model system for screening anticancer agents. In order to establish the usefulness of the nude mouse-human tumor xenograft system as a predictive screen for anticancer agents, it is necessary to demonstrate that clinically active (or inactive) drugs retain the same level of antineoplastic activity (or inactivity) against similar tumor types implanted as xenografts in athymic mice, as in the clinical patients.

The limited number of studies of antitumor drug activity on human tumors xenografted into athymic mice suggest that the response of such tumors to antitumor drugs is identical to the response of the patients of origin (22-24, 29, 33). We plan to test various clinically active drugs (in single and in combination

chemotherapy) against different types of human tumors which have been transplanted into athymic mice. To that end, we have studied the antitumor activity of DX<sup>4</sup> (Adriamycin), the most broad-spectrum single agent in cancer chemotherapy, against several human tumor xenografts, representative of some of the major classes of human cancer (breast, colon, lung, melanoma, ovary, prostate, sarcoma, and larynx).

The results of these studies indicate that the nude mouse-human tumor system offers a great potential for the identification of new anticancer agents of clinical interest.

## MATERIALS AND METHODS

**Athymic Mice.** All the animals used in these studies were 8- to 12-week-old female congenitally athymic BALB/c nude mice, homozygous for the *nu/nu* allele, bred in our laboratory. The colony of the mice was developed from breeding stock obtained from Laboratory Animal Breeding and Research Center G1, Ry, Denmark. As previously described (34, 35), the mice were maintained in isolation in autoclaved cages with polyester fiber filter covers, but not under germ-free or specific-pathogen-free conditions; all food, water, and bedding were sterilized. Every 3 months, for 3 weeks, piperazine hexahydrate, (1.3 mg/liter) was added to the drinking water to eliminate the intestinal nematodes normally present in mice, which might increase the T-cell-like activity in nude mice (2). At the end of the treatment, the mice were checked for the presence of further parasites.

**Tumors.** Human tumors, representative of some of the major classes of human cancer, were established by inoculation of fresh tumor tissue from patients into nude mice in our laboratory, as previously described (34, 35). Tumor tissue specimens obtained from the surgeon were transplanted into BALB/c *nu/nu* mice within 2 to 3 hr of resection. These specimens were rinsed with sterile medium containing antibiotics and then cut in small pieces for s.c. implantation. For serial transplantation, the tumor mass was removed under sterile conditions, separated from the capsule, minced in sterile medium containing antibiotics, and then, through two 18-gauge needles, inoculated s.c. on the backs of nude mice (0.2 ml/mouse). The number of previous passages ranged from 3 to 25 at the time of the study. The tumor take rate for all the tumor lines approached 90 to 100%, except for the lung tumor T 293, in which the take rate was approximately 40%. The donor patients had not been treated with DX before the surgery. Histological appearance of the serially transplanted tumors was identical to that of the original tumors. The growth curves of the studied tumors are shown in Chart 1, and the main characteristics of

<sup>1</sup>This work was supported in part by grants from the NIH, USPHS (CA-11683, 23052), NIH-NCI 5T32-CAO 3290-02; and the American Cancer Society (60).

<sup>2</sup>Supported in part by a contribution from Consiglio Nazionale delle Ricerche (CNR) of Italy. To whom requests for reprints should be addressed at: Division of Experimental Oncology B, Istituto Nazionale per lo Studio e la Cura dei Tumori, Venezian 1, 20133 Milan, Italy.

<sup>3</sup>National Research Service Award Training Fellow.  
 Received June 9, 1980; accepted October 3, 1980.

<sup>4</sup>The abbreviations used are: DX, doxorubicin; LDH, lactic dehydrogenase; T/C%, mean of relative tumor volume in treated mice/mean of relative tumor volume in control mice  $\times 100$ .

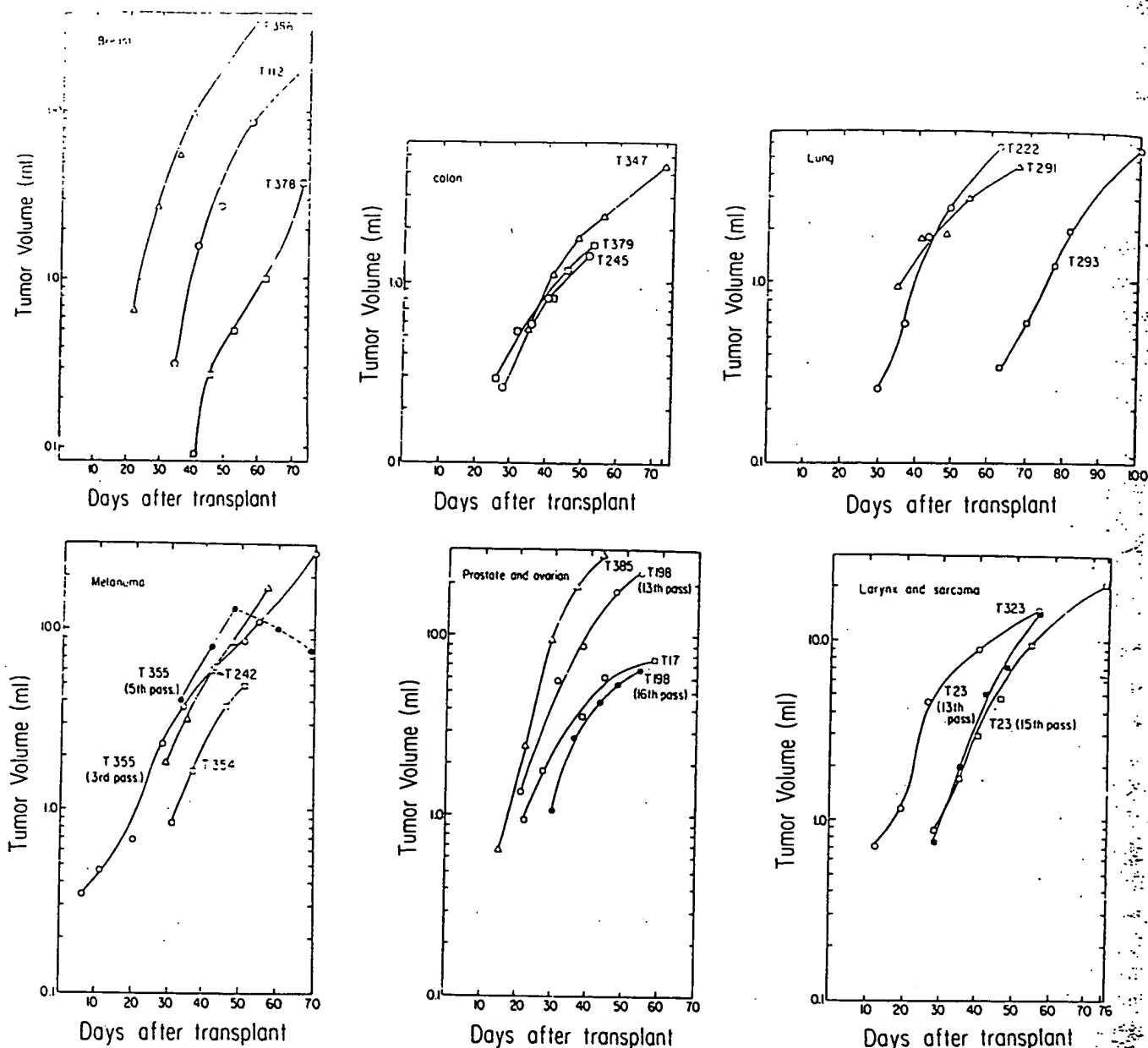


Chart 1. Growth of human tumors xenografted into nude mice. Each point, mean of 6 to 10 tumors.

these tumors are listed in Table 1. Individual growth of xenografts shows considerable variation (Chart 2); converting tumor volume measurements from absolute to relative values allows for easier visualization of antineoplastic activity of the drugs and standardized the tumor size at the start of the treatment.

**Tumor Quantity of LDH Isoenzyme.** The levels of mouse LDH and human LDH were quantitated in the tumor tissue transplanted into nude mice. Briefly, 4 tissue samples were taken, one from the tumor capsule, one from the portion immediately below the capsule, one from the core of the tumor, and one from the tissue between the capsule and the core. The tissue samples were homogenized separately and then centrifuged; the supernatants were collected and assayed for LDH activity using the pyruvate-to-lactate conversion procedure ( $\text{NADH} \rightarrow \text{NAD}^+$ ). Electrophoresis was carried out by determi-

nation of LDH isoenzymes in tumor supernatants using a Corning 72 fluorometer/densitometer. Human LDH standard was derived from human liver, and the mouse LDH standard was derived from corresponding BALB/c nude mouse liver. Twenty percent was arbitrarily fixed as the maximum allowable amount of mouse LDH in the tumor tissue sample that was taken from tumor core. The sample was not used if mouse LDH was more than 20%.

**Treatment and Evaluation of Chemotherapeutic Effect.** DX hydrochloride (supplied by Farmitalia Carlo Erba, Milano, Italy, and by Adria Laboratories, Columbus, Ohio) was dissolved in distilled water at a concentration such that the dose could be given in a volume of 0.1 ml/10 g body weight and was always administered i.v. weekly for 3 to 4 weeks. Because individual growth of implanted tumors shows great variability, we cannot

Table 1  
Characteristics of human tumors grown in athymic mice

Tumor	Tissue of origin	Histopathological characteristics	No. of passages <sup>a</sup>	% of mouse LDH activity <sup>b</sup>
T 112	Breast	Infiltrating and intraductal breast carcinoma	16	20
T 386	Breast	Infiltrating ductal carcinoma (pseudomedullary type)	6	0
T 378	Breast	Carcinoma with chondroid change	3	0
T 245	Lymph node	Adenocarcinoma (metastatic from colon)	12	16.5
T 347	Colon	Adenocarcinoma	4	7.5
T 379	Colon	Adenocarcinoma	3	9.3
T 222	Lung	Epidermoid carcinoma	21	14
T 291	Lung	Adenocarcinoma	11	5
T 293	Sternum	Oat cell carcinoma	18	5
T 242	Lymph node	Malignant melanoma	25	9
T 354 <sup>c</sup>	Melanoma	Amelanotic malignant melanoma	5	7
T 355 <sup>c</sup>	Melanoma	Malignant melanoma	3	10
			4	6
T 17	Ovary	Cystoadenocarcinoma	13	10
T 385	Ovary	Adenocarcinoma	3	0
T 198	Prostate	Adenocarcinoma	13	13
			16	0
T 23	Shoulder	Undifferentiated sarcoma	13	14
			15	4
T 323	Larynx	Well-differentiated epidermoid carcinoma with parakeratosis	10	10

<sup>a</sup> At the time of the experiment.

<sup>b</sup> The percentage of mouse LDH detected in the tumors at the time of the experiment (see "Materials and Methods").

<sup>c</sup> Both specimens obtained from a large tumor of the same patient.

a homogeneous size of tumor in the large number of mice in our experiments. Therefore, the treatment was started when transplant tumors became 0.5 to 1.5 cu cm. The DX was selected on the basis of toxicity studies performed in laboratory on nontumorous BALB/c nude mice.

The mice were randomized in groups of 6 to 10 animals each when the tumors reached palpable size. The tumors were measured weekly in 3 dimensions with a slide caliper. The tumor volume was calculated by the equation

$$\frac{1}{2} \pi d_1 \times d_2 \times d_3 = 1.57 \times d_1 \times d_2 \times d_3$$

where  $d_1$ ,  $d_2$ , and  $d_3$  are the experimental measurements (in mm) of tumor length, width, and thickness. Each tumor volume was then expressed in relative tumor volume (RV) by the formula

$$RV = V_x / V_i$$

where  $V_x$  is the volume at any given day and  $V_i$  is the tumor volume at the start of treatment. The T/C% value was calculated each time that the tumors were measured; the lowest T/C% was expressed as an optimal T/C% for each group. Each time the tumors were measured, control relative tumor values were compared with treated relative tumor volumes by Student's *t* test.

## RESULTS AND DISCUSSION

Seventeen human tumors serially transplanted in athymic mice (3 breast tumors, T 112, T 378, and T 386; 3 colon tumors, T 245, T 347, and T 379; 3 lung tumors, T 222, T 291, and T 293; 3 melanomas, T 242, T 354, and T 355; 2 ovary tumors, T 17 and T 385; one prostate tumor, T 198; one sarcoma, T 23; and one larynx tumor, T 323) were tested for sensitivity to DX. The experimental results obtained were compared with clinical response of the above tumors to DX in humans, as reported in the literature. As discussed below, the

activity of DX against all the tumors studied, except the melanomas, is in good agreement with the clinical data. The results are briefly discussed here, divided according to the different classes of tumors studied, and are given in Tables 2, 3, and 4.

**Breast Cancer.** Clinically, breast cancer is responsive to a wide range of single agents, including DX (8). As a single agent, DX has a 40 to 50% response rate in metastatic breast cancer in patients previously untreated with chemotherapy (6, 9). In the nude mouse-human system, DX was tested on 3 breast tumors originating from 3 different patients and produced a delay in the tumor growth and a statistically significant decrease in the tumor volume in all 3 (Chart 3; Table 3). In case of breast tumor T 112, 2 dose schedules of DX were tested, 6 and 10 mg/kg/injection. Both the dose schedules produced statistically significant decreases in the tumor volume (T/C% = 6.9 and 11.8, respectively). The difference in activity of the low and high dose levels (6 and 10 mg/kg/injection) could be due to the fact that the 6-mg/kg group was inadvertently treated with 15 instead of 6 mg DX per kg per injection in the first treatment. This is probably the reason for the more effective inhibition of the tumor growth in the 6-mg DX per kg per injection dosage group. Sensitivity of breast tumors T 386 and T 378 to 10 mg DX per kg per injection every 7 days for 3 doses was also statistically significant (T/C% = 3.8 and 10.5, respectively). Chart 4 shows, in an illustrative experiment, the relative growth curves graphed for each individual mouse in the control groups and in the treated groups. The xenografts show considerable variations in both the controls and the treated groups. The individual curves show that the response of the tumors to DX treatment follows the same patterns in all the treated animals and the shape of the curves is parallel.

**Colon Tumors.** The majority of single agents are ineffective against colorectal tumors and those which are effective are only marginally so (5, 8). Among the useful drugs, DX is not included (5, 18). Three different human colon tumors transplanted into nude mice were used to test the DX activity. Chart

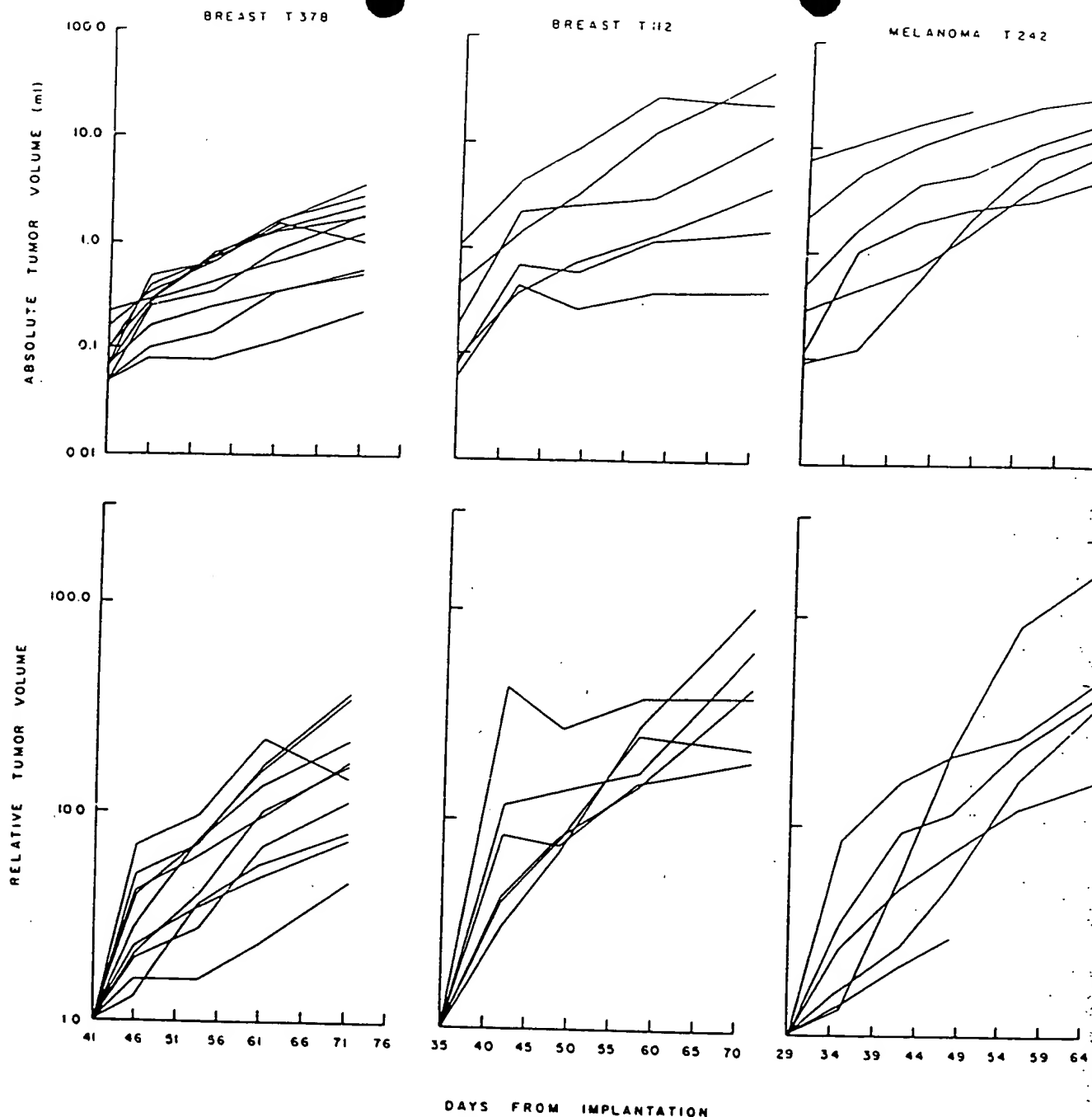


Chart 2. Absolute and relative tumor volume for human breast tumors T 112, T 378, and melanoma T 242. Each curve represents the growth of a tumor in an individual mouse.

5 and Table 3 show the results; DX administered i.v. at a dose of 10 mg/kg/injection every 7 days for 3 doses did not show any effect on the tumor growth. In other experiments involving screening of DX against 7 different colorectal tumors, we have observed similar results (26).

**Lung Tumors.** In a recent review of the literature on the use of DX as a single agent on lung cancer, Selawry (37) reported an overall response rate of 21% in 321 patients. The main histological types of bronchogenic carcinoma (epidermoid carcinoma, adenocarcinoma, and large- and small-cell carcinoma)

respond differently to different antitumor drugs (12, 16, 37). Epidermoid carcinoma, the most common cell type (37), has an objective response to DX between 19% (37) and 35% (5); for small-cell carcinoma, the objective response is 25% (4, 16). The remaining cell types, adenocarcinoma and large-cell carcinoma, seem to be less responsive to DX (5). Chart 6 and Table 3 show the results of the chemotherapy performed with 3 different types (T 222 epidermoid carcinoma, T 291 adenocarcinoma, and T 293 oat cell carcinoma) of human lung tumors transplanted into nude mice. A statistically significant

Table 2  
Experimental chemotherapy of human tumors heterotransplanted into nude mice

Tumor	T/C% after following doses of DX			
	4.4 mg/kg/injection	6 mg/kg/injection	6.6 mg/kg/injection	10 mg/kg injection
Breast				
T 112		+++ <sup>a, b</sup>		--- <sup>c</sup>
T 378				+++ <sup>c</sup>
T 386				+++ <sup>c</sup>
Colon				
T 245				-
T 347				-
T 379				-
Lung				
T 222			++ <sup>a</sup>	
T 291		-		+++ <sup>c</sup>
T 293				
Melanoma				
T 242		+		--- <sup>c</sup>
T 354				+++ <sup>c</sup>
T 355 <sup>d</sup> (3rd passage)		+++ <sup>c</sup>		+++ <sup>c</sup>
T 355 (4th passage)		±		+++ <sup>c</sup>
Ovary				
T 17		-		±
T 385				-
Prostate				
T 198 (13th passage)		-		+++ <sup>c</sup>
T 198 (15th passage)				+++ <sup>c</sup>
Sarcoma				
T 23 (13th passage)	+		+++ <sup>c</sup>	+++ <sup>c</sup>
T 23 (15th passage)				+++ <sup>c</sup>
Larynx				
T 323		-		-

<sup>a</sup> Three i.v. treatments, one each week, starting when the tumor volume became relatively large.

<sup>b</sup> -, T/C% higher than 50%; ±, T/C% close to 50%; +, T/C% from 35 to 50%; ++, T/C% from 20 to 35%; ++++, T/C% lower than 20%; +++++, regression of the tumor to a volume smaller than the volume at the start of treatment.

<sup>c</sup> Statistically significant as evaluated by Student's *t* test.

<sup>d</sup> Treatment started 6 days after tumor transplantation.

egression in the growth of the T 293 oat cell carcinoma (10 mg/kg/injection every 7 days for 3 doses; T/C% < 1,  $p < 0.01$ ) and T 222 epidermoid carcinoma (6.6 mg/kg/injection every 7 days for 3 doses; T/C% = 20,  $p < 0.02$ ) was produced by DX injections. A slight and not statistically significant response was obtained on T 291 adenocarcinoma.

**Malignant Melanoma.** DX is inactive in the treatment of human metastatic melanoma (4, 39). We tested the DX activity in 3 different human melanomas, derived from 2 different patients (Chart 7; Table 3). Treatment of T 242, a malignant melanoma that has metastasized to a lymph node, was started when the tumors were in the advanced stage and produced a dose-dependent delay in tumor growth (T/C% = 13,  $p < 0.05$ ). The chemotherapy of melanotic melanoma T 355 either was given 6 days after the tumor implantation (early-stage tumor treatment), or was delayed until the tumor became relatively large (advanced stage of tumor treatment). The early-stage tumor was more sensitive than the advanced-stage tumor. Both the doses tested gave a dose-dependent statistically significant reduction in tumor growth, in early-stage tumors (6 mg/kg/injection: T/C% = 32.7,  $p < 0.01$ ; 10 mg/kg/injection: T/C% = 7.8,  $p < 0.005$ ); whereas in the advanced stage of tumors, the DX activity was detected only at the higher dose level (10 mg/kg/injection: T/C% = 48.9,  $p < 0.02$ ). DX was not active against T 354 tumor.

The DX activity against the melanomas tested is quite surprising. The DX activity against the T 355 melanoma treated in the early stage could be explained on the basis that the growth fraction is larger, the vascular supply is better, and the response to the drug is better in small tumors (3, 20), but it is difficult to explain the experimental activity of DX on large tumors. Bellet *et al.* (3) have reported that 7 established human melanoma tissue-cultured cell lines heterotransplanted in nude mice and treated with DX were refractory to the treatment. However, Karakousis (30) has recently shown that high levels of DX produced by tourniquet infusion can cure human extremity melanomas. Two of the melanomas tested, T 354 and T 355, although obtained from a large tumor from the same patient, differ in their histological characteristics and also differ in their response to DX treatment. This suggests that heterogeneity of tumors could be a major cause of little sensitivity of some of the tumors to monotherapy and good response to combination chemotherapy.

**Ovarian Tumors.** A variety of drugs are active against ovarian cancer (15). DX as single agent is a useful drug in the treatment of ovarian carcinoma, in both previously untreated patients and in those who have failed to respond to alkylating agent therapy (5, 11, 15, 18). DX has a 33 to 38% overall response rate in ovarian cancer (1, 19). The experimental activity of DX tested against 2 different ovarian tumors (T 17

Table 3

Effect of DX on human breast, colon, lung, melanoma, and ovary tumors transplanted into nude mice<sup>a</sup>

Tumor	Dose (mg/kg/injection)	DTS <sup>b</sup>	Optimal T/C%	p <sup>c</sup>
Breast				
T 112 <sup>d</sup>	6	34	6.9 (23) <sup>e</sup>	<0.01
	10		11.8 (23)	<0.01
T 386	10	21	3.8 (40)	<0.01
T 378	10	37	10.5 (20)	<0.01
Colon				
T 245	10	60	99 (8)	
T 347	10	34	90 (7)	
T 379	10	40	81 (15)	
Lung				
T 222	4.4	30	74.7 (19)	
	6.6		20 (19)	<0.02
T 291	6	35	70.4 (6)	
	10		77.9 (32)	
T 293	10	64	<1 <sup>f</sup> (18)	<0.01
Melanoma				
T 242	6	29	49 (27)	
	10		13 (19)	<0.05
T 355 <sup>g</sup>	6	6	32.7 (22)	<0.01
	10		7.8 (39)	<0.005
T 355	6	32	61 (9)	
	10		48.9 (15)	<0.02
T 354	10	29	44 (20)	
Ovary				
T 17	6	22	67.5 (36)	
	10		57.8 (36)	
T 385	10	16	47.6 (30)	

<sup>a</sup> Three i.v. treatments, one each week. Six to 10 mice were used in each experiment for both control and treated groups.<sup>b</sup> DTS, days after tumor transplant that the treatment was started.<sup>c</sup> Analysis performed between controls versus treated groups using Student's *t* test.<sup>d</sup> The 6-mg/kg dosage group was inadvertently given an initial dose of 15 mg/kg/injection.<sup>e</sup> Numbers in parentheses, day of evaluation after the start of treatment.<sup>f</sup> Tumor volume became less than the initial tumor volume (regression).<sup>g</sup> Treatment started 6 days after tumor transplantation. The dose schedule used was every 7 days for 4 doses.

Table 4

Effect of DX on human prostate, sarcoma, and larynx

Tumor	No. of transfers	Schedule of treatment <sup>a</sup>	Dose (mg/kg/injection)	DTS <sup>b</sup>	Optimal T/C%	p <sup>c</sup>
Prostate	13					
T 198		q7d x 4 <sup>d</sup>	6	21	62.4	
	16		10		11.6	<0.01
		q7d x 3	10	30	31.7	<0.01
Sarcoma	13					
T 23		q7d x 4	4.4	28	38.9	
	15		6.6		19.8	<0.05
		q7d x 3	10	29	16.5	<0.01
Larynx	10					
T 323		q7d x 3	6		64.5	
			10	30	40.3	

<sup>a</sup> Three i.v. treatments, one each week. Six to 10 mice were used in each experiment for both control and treated groups.<sup>b</sup> DTS, days after tumor transplant that the treatment was started.<sup>c</sup> Analysis performed between controls versus treated groups using Student's *t* test.<sup>d</sup> q7d x 3 or 4, every 7 days for 3 or 4 doses.

and T 385) transplanted into nude mice is shown in Chart 8 and in Table 3. The activity was slight and not statistically significant: T 17, T/C% = 57.8; and T 385, T/C% = 47.6.

**Prostate, Sarcoma, and Larynx Tumors.** Prostate cancer

treatment is normally performed by surgery, radiation, and hormonal therapy in different sequences. The objective response rate to DX is 26 to 29% (21, 28, 31) in patients with prostate carcinoma who have failed treatment with hormones. DX was tested against prostate adenocarcinoma T 198 transplanted into nude mice in 2 different experiments (Chart 9; Table 4) involving tumors with different passage numbers, and it produced a delay in the tumor growth in both [T 198 (13th passage), 10 mg/kg/injection: T/C% = 11.6,  $p < 0.01$ ; and 6 mg/kg/injection: T/C% = 62.4]. In another experiment performed with T 198 tumor, the response to DX treatment (10 mg/kg/injection) was slightly less than in the previous experiment (T/C% = 31.7,  $p < 0.01$ ).

The first really useful drug found to be active against human adult soft tissue sarcomas was DX (7, 32); the response rate to DX of soft-tissue sarcoma is about 30% (7, 28, 32).

The DX treatment against T 23 human soft-tissue sarcoma transplanted into athymic mice was performed with 2 different schedules in 2 different experiments. DX administered at the dose of 6.6 mg/kg/injection twice a week for 2 weeks (Chart 10; Table 4) produced a dose-dependent statistically significant delay in the tumor growth: T/C% = 19.8,  $p < 0.05$ . In another experiment with T 23 sarcoma, administration of DX once a week for 3 weeks at the dose of 10 mg/kg/injection produced a statistically significant delay in the tumor growth: T/C% = 16.5,  $p < 0.01$ .

DX is active against head and neck carcinoma, but the literature data are generally expressed as an overall rate,

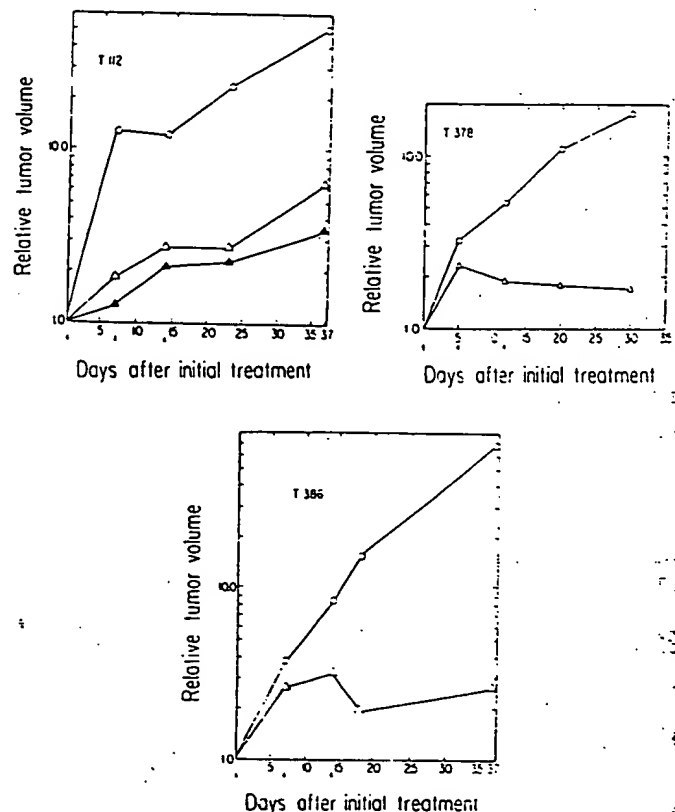


Chart 3. Response of T 112, T 386, and T 378 human breast tumors in nude mice to DX. O, controls; Δ, 10 mg/kg/injection; ▲, 6 mg/kg/injection. Relative tumor volume is the ratio of tumor volume at any given day to the tumor volume when the treatment was started. Arrows, treatment.

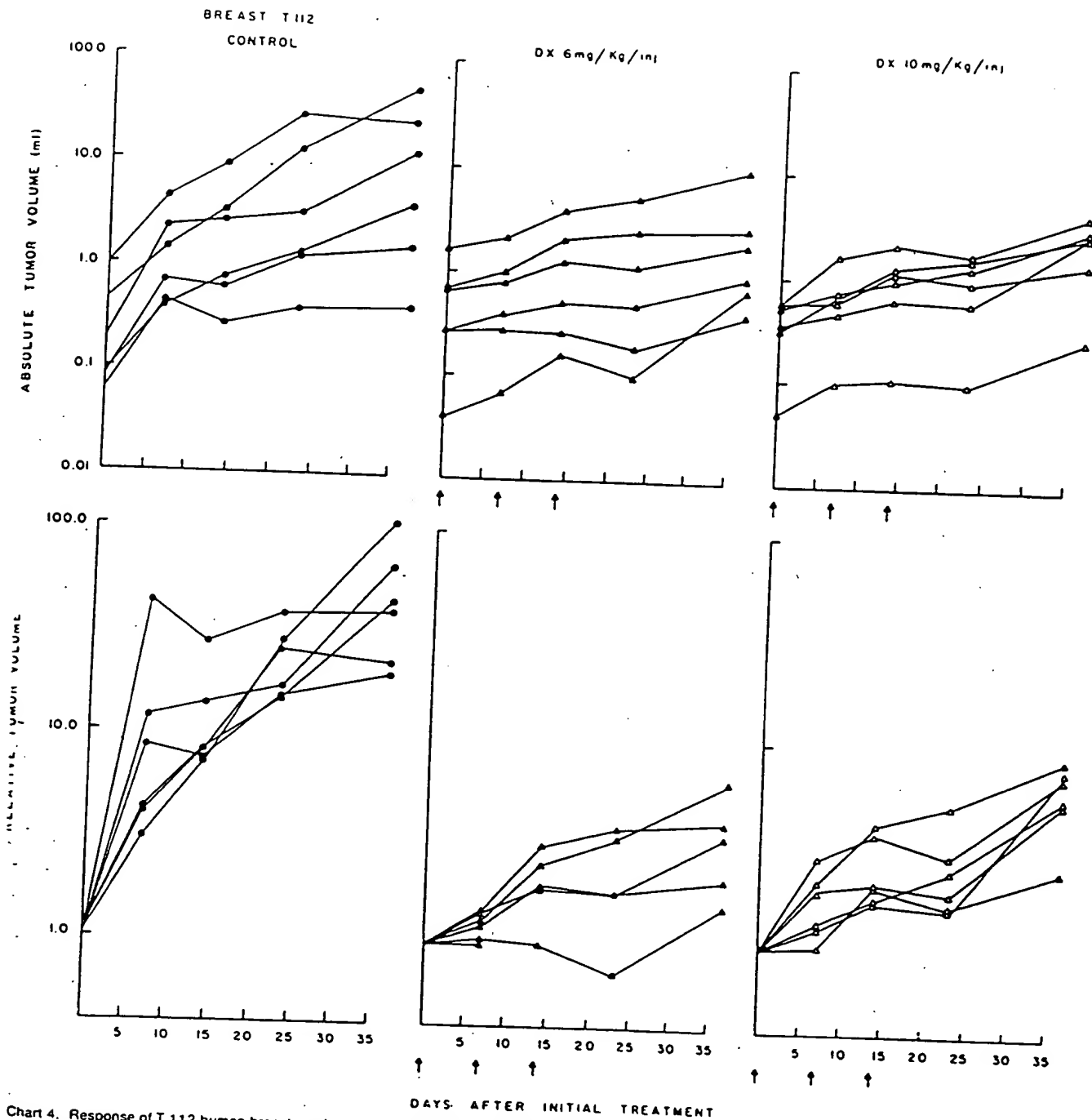


Chart 4. Response of T 112 human breast carcinoma in nude mice to DX reported as absolute and relative tumor volume. Each curve represents the growth of a tumor after the i.v. treatment in an individual mouse. Arrows, treatment.

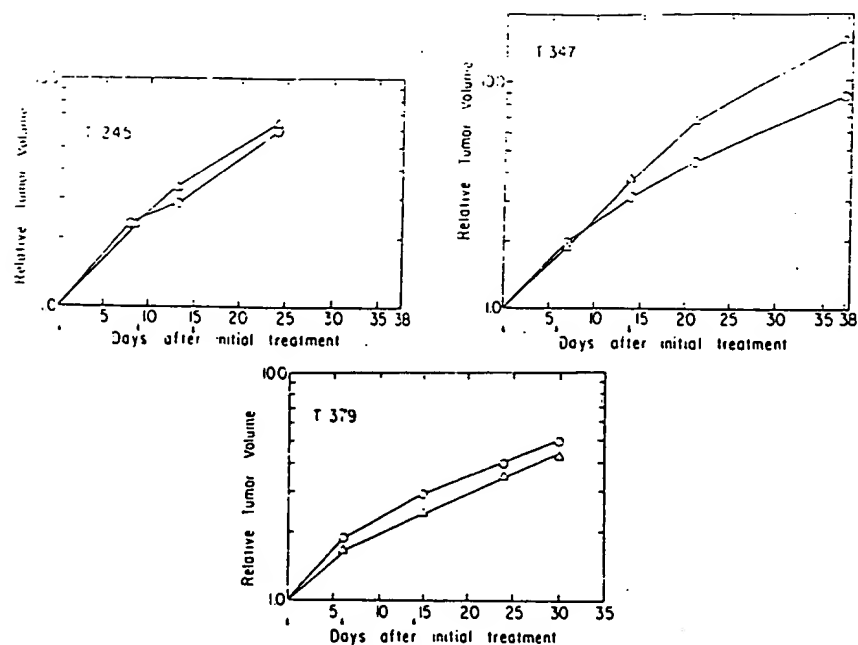


Chart 5. Response of T 245, T 379, and T 347 human colon tumors in nude mice to DX. O, controls; Δ, 10 mg/kg/injection. Arrows, treatment.

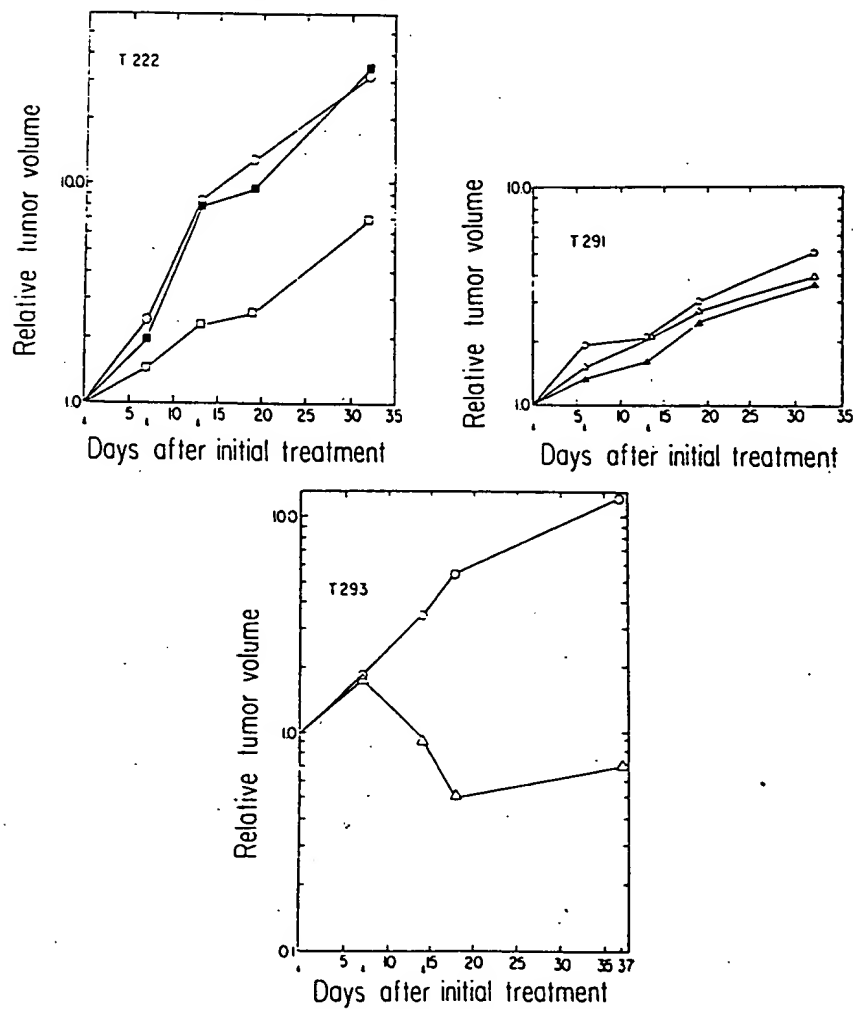


Chart 6. Response of T 222, T 291, and T 293 human lung tumors in nude mice to DX. O, controls; ■, 4.4 mg/kg/injection; ▲, 6 mg/kg/injection; □, 6.6 mg/kg/injection; Δ, 10 mg/kg/injection. Arrows, treatment.



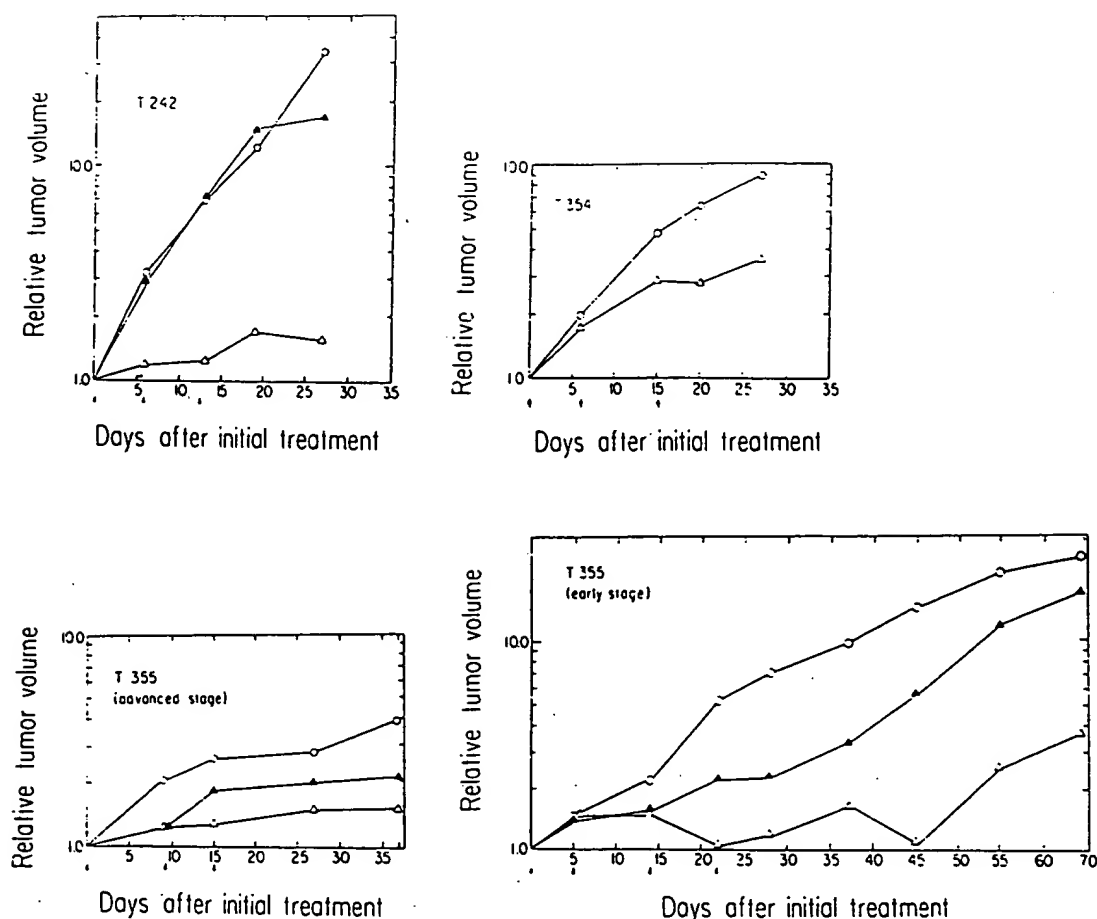


Chart 7. Response of T 242, T 354, and T 355 human melanomas in nude mice to DX. O, controls; ▲, 6 mg/kg/injection; △, 10 mg/kg/injection. Arrows, treatment.

noting the heterogeneity of sites that comprise this group of tumors. However, of 7 single-agent chemotherapeutic drugs active against common head and neck cancer, DX has the best response percentage, 23% (10, 17, 27). In agreement with the clinical activity in our experiments, the antitumor activity (Chart 11; Table 4) of DX on the growth of human larynx epidermoid carcinoma T 323 implanted into nude mice was slight and not statistically significant.

The high experimental antitumor activity of DX has been related to the host immunological response (13, 14, 25). It was found that DX, compared to daunorubicin, has a lower effect on the cellular compartment which is responsible for cell-mediated immunity (T-cells) than on the cellular compartment responsible for synthesis of antibodies (B-cells) (14, 36). The DX activity against tumors transplanted into athymic mice appears to alter the significance of these results. Experiments are in progress to test the daunorubicin activity against human tumors growing in athymic mice with the aim to clarify the problem.

## CONCLUSIONS

From the above studies the following conclusions can be

drawn: (a) the results of DX chemotherapy studies on a panel of human tumors xenografted into nude mice reflect the clinical response rates very closely, except in the case of the activity of DX against the melanomas and ovarian cancer; (b) the activity of DX differs when used against lung tumors of different histological types but is closely related to the clinical activities; and (c) the response of DX of the 2 (T 23 and T 198) tumors tested at the different passages was essentially the same.

Because human tumors of the same type exhibit a wide variability, their response to the anticancer drugs both in patient and in xenografts, the chemosensitivity of human tumors cannot be evaluated on the basis of the sensitivity of a few xenografts. Therefore, information about activity of antitumor drugs (old or new) can be best obtained by testing the drugs against a panel of tumors. Consequently, we are increasing the tumor numbers representing the major classes of human neoplasms in stock in our facility (actually 39).

The results indicate that there is a good correlation between the sensitivity of tumors to a drug in the human body and in BALB/c nude mice. Therefore, it is reasonable to assume that the human tumor-nude mouse system may be a suitable model for selection of drugs for the treatment of human cancer and for screening of new antitumor agents.

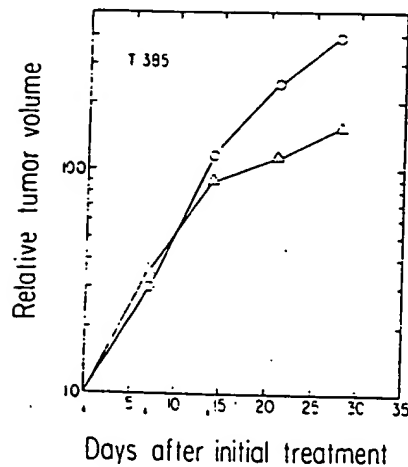
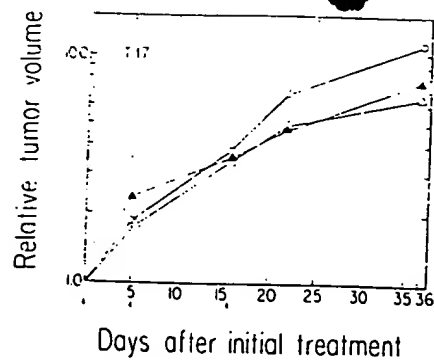


Chart 8. Response of T 17 and T 385 human ovarian tumors in nude mice to DX. O, controls; ▲, 6 mg/kg/injection; Δ, 10 mg/kg/injection. Arrows, treatment.

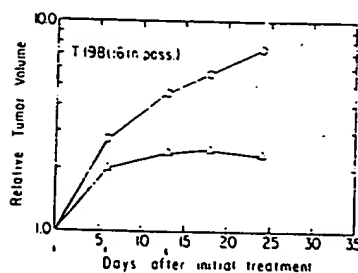
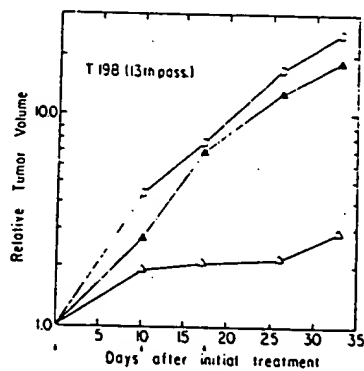


Chart 9. Response to DX of T 198 human prostate tumor in its 13th and 16th passages transplanted into nude mice. O, controls; ▲, 6 mg/kg/injection; Δ, 10 mg/kg/injection. Arrows, treatment.

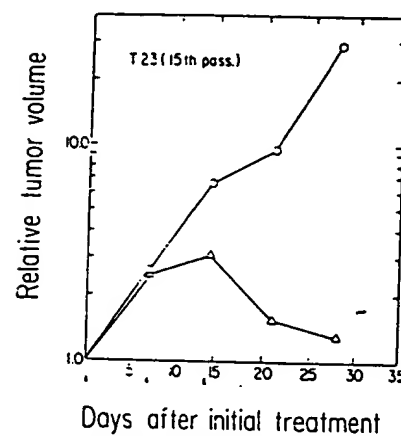
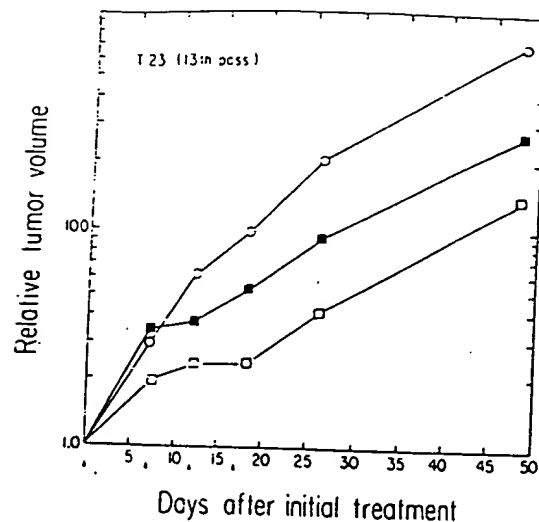


Chart 10. Response to DX of T 23 human sarcoma in its 13th and 15th passages transplanted into nude mice. O, controls; ▲, 4.4 mg/kg/injection; □, 6.6 mg/kg/injection; Δ, 10 mg/kg/injection. Arrows, treatment.

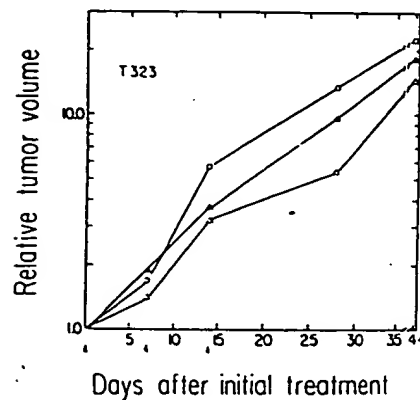


Chart 11. Response to DX of T 323 human larynx tumor transplanted into nude mice. O, controls; ▲, 6 mg/kg/injection; Δ, 10 mg/kg/injection. Arrows, treatment.

## ACKNOWLEDGMENTS

For excellent technical assistance, we wish to thank R. Rice, B. Wolf, and A. Coirin. We are grateful to Drs. S. Baird and E. Elson for histological analysis.

## REFERENCES

- Anderson, T., and Young, R. C. Recent advances in the staging and treatment of ovarian cancer. *Med. Clin. N. Am.*, 61: 1001-1012, 1977.
- Beattie, G., Lipsick, J., Lannon, R., Baird, S., Kaplan, N. O., and Osler, G. Induction of T cell-like response in athymic mice. In: *Proceedings of the Third International Workshop on Nude Mice*, Bozeman, Mont., September 6-9, 1979, in press, 1981.
- Bellet, R. E., Danna, V., Mastrangelo, M., and Berd, D. Evaluation of a "nude" mouse-human tumor panel as a predictive secondary screen for cancer chemotherapeutic agents. *J. Natl. Cancer Inst.*, 63: 1185-1188, 1979.
- Bellet, R. E., Mastrangelo, M., Berd, D., and Lustbader, E. Chemotherapy of metastatic malignant melanoma. In: W. H. Clark, Jr., Goldman, and M. Mastrangelo (eds.), *Human Malignant Melanoma*, pp. 325-351. New York: Grune & Stratton, 1979.
- Blum, R. H., and Carter, S. K. Adriamycin. A new anticancer drug with significant clinical activity. *Ann. Intern. Med.*, 80: 249-259, 1974.
- Bonadonna, G., Beretta, G., Tancini, G., Brambilla, C., Bajetta, G., DePalo, M., De Lena, M., Fossati-Bellani, M., Gasparini, P., Valagussa, P., and Veronesi, U. Adriamycin (NSC-123127) studies at the Istituto Nazionale Tumori, Milan. *Cancer Chemother. Rep.*, 6 (Part 3): 231-245, 1975.
- Bonadonna, G., Monfardini, S., DeLena, M., Fossati-Bellani, F., and Beretta, G. Phase I and preliminary phase II evaluation of adriamycin (NSC-123127). *Cancer Res.*, 30: 2572-2582, 1970.
- Carter, S. K. Current protocol approaches in large bowel cancer. *Semin. Oncol.*, 3: 433-443, 1976.
- Carter, S. K. Integration of chemotherapy into combined modality treatment of solid tumors. VII. Adenocarcinoma of the breast. *Cancer Treat. Rev.*, 3: 141-179, 1976.
- Carter, S. K. The chemotherapy of head and neck cancer. *Semin. Oncol.*, 4: 413-424, 1977.
- Carter, S. K., Bakowski, M. T., and Hellmann, K. Gynecologic cancer. In: S. K. Carter and M. Bakowski (eds.), *Chemotherapy of Cancer*, pp. 138-158. New York: John Wiley & Sons, 1977.
- Carter, S. K., Bakowski, M. T., and Hellmann, K. Bronchogenic carcinoma. In: S. K. Carter and M. Bakowski, (eds.), *Chemotherapy of Cancer*, pp. 193-199. New York: John Wiley & Sons, 1977.
- Casazza, A. M., Di Marco, A., and Di Cunzio, G. Interference of daunomycin and adriamycin on the growth and regression of murine sarcoma virus (Moloney) tumors in mice. *Cancer Res.*, 31: 1971-1976, 1971.
- Casazza, A. M., Isetta, A. M., Giuliani, F., and Di Marco, A. Immunodepressive activity of Daunomycin and Adriamycin. In: M. Staguët, H. Tagnon, Y. Kemis, et al. (eds.), *Adriamycin Review*, pp. 123-131. Ghent, Belgium: European Press Medikon, Part II, 1975.
- Cline, J. M., and Haskell, C. M. Genitourinary neoplasia. In: J. M. Cline and C. M. Haskell (eds.), *Cancer Chemotherapy*, pp. 112-155. W. B. Saunders Co., Philadelphia: 1980.
- Cline, J. M., and Haskell, C. M. Lung Cancer. In: J. M. Cline and C. M. Haskell (eds.), *Cancer Chemotherapy*, pp. 175-186. Philadelphia: W. B. Saunders Co., 1980.
- Cline, J. M., and Haskell, C. M. Cancer of the head and neck. In: J. M. Cline and C. M. Haskell (eds.), *Cancer Chemotherapy*, pp. 187-198. Philadelphia: W. B. Saunders Co., 1980.
- Davis, H. L., and Davis, T. E. Daunorubicin and Adriamycin in cancer treatment: an analysis of their roles and limitations. *Cancer Treat. Rep.*, 63: 809-815, 1979.
- DeVita, V. T., Wasserman, T. H., Young, R. C., and Carter, S. K. Perspective on research in gynecologic oncology. *Treatment protocols. Cancer (Phila.)*, 38: 503-525, 1976.
- Donelli, M. G., Colombo, T., Broggetti, M., and Garattini, S. Differential distribution of anti-cancer agents in primary and secondary tumors. *Cancer Treat. Rep.*, 61: 1319-1324, 1977.
- Eagan, R. T., Hahn, R. G., and Myers, R. P. Adriamycin versus 5-fluorouracil and cyclophosphamide in the treatment of metastatic prostate cancer. *Cancer Treat. Rep.*, 60: 115-117, 1976.
- Giovanella, B. C., and Fogh, J. Present and future trends in investigations with the nude mouse as a recipient of human tumor transplant. In: J. Fogh, and B. C. Giovanella (eds.), *The Nude Mouse in Experimental and Clinical Research*, pp. 281-312. New York: Academic Press, Inc., 1978.
- Giovanella, B. C., Stehlin, J. S., Fogh, J., and Sharkey, F. E. Serial transplantation of human malignant tumors in nude mice and their use in experimental chemotherapy. In: D. P. Houchens and A. A. Ovejera (eds.), *Proceedings of Symposium on the Use of Athymic (Nude) Mice in Cancer Research*, pp. 163-179. Stuttgart: Gustav Fischer Verlag, 1978.
- Giovanella, B. C., Stehlin, J. S., Shepard, R. C., and Goldin, A. Experimental chemotherapy of human malignant tumors serially heterotransplanted in nude mice. *Proc. Am. Assoc. Cancer Res.*, 18: 27, 1977.
- Giuliani, F., Casazza, A. M., and Di Marco, A. Combined immunotherapy and chemotherapy of Moloney sarcoma virus induced tumors in mice. *Biomedicine*, 18: 387-392, 1973.
- Giuliani, F. C., and Kaplan, N. O. New doxorubicin analogs active against doxorubicin resistant colon tumor xenografts in the nude mouse. *Cancer Res.*, 40: 4682-4687, 1980.
- Goldsmith, M. A., and Carter, S. K. The integration of chemotherapy into a combined modality approach to cancer therapy. V. Squamous cell cancer of the head and neck. *Cancer Treat. Rev.*, 2: 137-158, 1975.
- Gottlieb, J. A., Baker, L. H., O'Bryan, R. M., Sinkovics, J. G., Hoogstraten, B., Ouagliana, J. M., Riukin, S. E., Bodey, G. P., Sr., Rodriguez, V. T., Blumenschein, G. R., Saiki, J. H., Colman, C., Jr., Burgess, M. A., Sullivan, P., Thigpen, T., Bottomley, R., Balcerzak, S., and Moon, T. E. Adriamycin used alone and in combination for soft tissue and bony sarcomas. *Cancer Chemother. Rep.*, 6 (Part 3): 271-282, 1975.
- Houchens, D. P., Ovejera, A. A., and Baker, A. D. The therapy of human tumors in athymic (nude) mice. In: D. P. Houchens and A. A. Ovejera (eds.), *Proc. Symposium on the Use of the Athymic (Nude) Mice in Cancer Research*, pp. 267-280. Stuttgart: Gustav Fischer Verlag, 1978.
- Karakousis, C. P. Tourniquet infusion (T.I.) versus perfusion (P) of extremities with Adriamycin (Am). *Proc. Am. Assoc. Cancer Res.*, 21: 157, 1980.
- O'Bryan, R. M., Baker, L. H., Gottlieb, J. A., Riukin, S. E., Blacierzak, S. P., Grumet, G. N., Salmon, S. E., Raoun, T. E., and Hoogstraten, B. Dose response evaluation of Adriamycin in human neoplasia. *Cancer (Phila.)*, 38: 1940-1948, 1977.
- O'Bryan, R. M., Luce, J. K., Talley, R. W., Gottlieb, J. A., Baker, L. M., and Bonadonna, G. Phase II evaluation of Adriamycin in human neoplasia. *Cancer (Phila.)*, 32: 1-8, 1973.
- Povlsen, C. O., and Jacobsen, G. K. Chemotherapy of a human malignant melanoma transplanted in the nude mouse. *Cancer Res.*, 35: 2790-2796, 1975.
- Reid, L., Colburn, P., Sato, G., and Kaplan, N. O. Approaches to chemotherapy using the athymic nude mouse. In: D. P. Houchens and A. A. Ovejera (eds.), *Symposium on the Use of Athymic (Nude) Mice in Cancer Research*, pp. 123-132. Stuttgart: Gustav Fischer Verlag, 1978.
- Reid, L., and Seung-il-Shin. Transplantation of heterologous endocrine tumor cells in nude mice. In: J. Fogh and B. C. Giovanella (eds.), *The Nude Mouse in Experimental and Clinical Research*, pp. 313-351. New York: Academic Press, Inc., 1978.
- Schwartz, H. S., and Grindey, G. B. Adriamycin and daunomycin: a comparison of antitumor activity and tissue uptake in mice following immunosuppression. *Cancer Res.*, 33: 1837-1844, 1973.
- Selawry, O. S. The role of chemotherapy in the treatment of lung cancer. *Semin. Oncol.*, 1: 259-272, 1974.
- Selby, P., Thomas, J. M., and Peckham, M. A comparison of the chemosensitivity of a primary tumor and its metastases using a human tumor xenograft. *Eur. J. Cancer*, 15: 1425-1429, 1979.
- Sieper, W., Mastrangelo, M., and Bellet, R. E. Phase II study of adriamycin (NSC-123127) in patients with metastatic melanoma. *Cancer Chemother. Rep.*, 59: 1181-1182, 1975.

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☒ ~~BLACK BORDERS~~

☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES

☐ FADED TEXT OR DRAWING

☒ ~~BLURRED OR ILLEGIBLE TEXT OR DRAWING~~

☐ SKEWED/SLANTED IMAGES

☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS

☐ GRAY SCALE DOCUMENTS

☐ LINES OR MARKS ON ORIGINAL DOCUMENT

☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY

☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**